Numbering of Claims

Detailed Office Action

1. The originally submitted claims contained two consecutive claims numbered as 27. The later of the claims was renumbered as 28, and the remaining claims were renumbered as 29 to 54, respectively. Claims 1-54 are pending and examined on the merits.

Response:

In accordance with 37 CFR §1.126, the original numbering of the claims will be preserved throughout prosecution, but the applicants agree with the changes to the numbering, resulting from the redundant numbering of claim 27. All claim related references in the applicants' response will refer to examiner's nomenclature for examination purposes and will be referenced according to the examiner's renumbered format.

PRIORITY

Detailed Office Action

2. Applicant's claim for priority to application number 09/248,388 and provisional applications 60/074,535, 60/110,279 and 60/110,202 is acknowledged. However, the non-provisional and provisional applications upon which priority is claimed fails to provide adequate support under 35 U.S.C. 112 for the instant invention as no mention of the stabilized cells of the instant invention has been made in any of the prior applications. The priority date for the instant application will be given the instant filing date of March 7, 2001.

Response:

The applicants respectfully suggest that the relevant inquiry in determining 35 USC § 112 is whether the claims set out and circumscribe a particular area with a reasonable degree of precision and particularity such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (CCPA 1971). Any consideration should be in light of the teachings of the prior art and of the particular application disclosure, as interpreted by one having ordinary skill in the art. In re Moore, supra.

Thus while a claim for priority may exist with a specific reference to stabilized cells of the instant invention, priority should also exist for claims supported by references to aspects of the instant invention encompassing the metes and bounds as interpreted by one having ordinary skill in the art. Application number 09/248,388 (now US 6,365,362) and provisional applications 60/074,535, 60/110,279 and 60/110,202 describe the two stage methodology for enrichment of target bioentities combined with multiparameter flow cytometric, microscopic and immunocytochemical analysis. One having ordinary skill in the art would realize the requirement for having control cells for the isolation and identification of rare cells. Further, claims to the labeling of cells in the present invention, albeit not fixed, stabilized, or otherwise, are specifically mentioned (see US 6,365,362, column 12, line 67 to column 13, line 67 and see column 15, line 54 to column 16, line 29). Plus, an internal control cell was used in assessing EpCAM antibody efficiency (see US 6,365,362, column 24, table II).

Accordingly, the applicants respectfully request the examiner's reconsideration and withdrawal of this objection to applicant's claim of priority and reinstatement of the earlier claimed priority, it being respectfully submitted that the applications having the earlier priority dates teach the use of internal control cells, as would be apparent to one of ordinary skill in the art.

SPECIFICATION

Detailed Office Action

3. The disclosure is objected to because of the following informalities: The specification claims priority to application 09/248,388, which is not a parent application as it fails to disclose or mention the subject matter of the instant invention.
Appropriate correction is required.

Response:

Applicants respectfully refer to above mentioned **Priority** arguments. Applicants further request the opportunity to rectify this objection in the manner suggested by the examiner should the earlier priority not be reinstated as requested by applicants above.

OATH/DECLARATION

Detailed Office Action:

4. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Priority to claimed to applications, which the instant application is not entitled for the reasons set forth above.

Response:

Applicants respectfully refers the examiner to **Priority** arguments set forth above. Applicants further request the opportunity to rectify the objection in the manner suggested by examiner should the earlier priority not be reinstated as requested by applicants above.

CLAIM REJECTIONS – 35 USC § 112, SECOND PARAGRAPH

Detailed Office Action

7. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Detailed Office Action

8. Claims 1-54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 7 recites "Physically and biologically stable". The specification defines "stabilized" a maintaining antigen integrity in a reproducible manner over time. The

specification does not define "physically and biologically stable" versus stabilized". For purpose of examination "biologically stabilized" will be read as maintaining antigen integrity and cellular integrity over time.

Claims 5, 15 and 32 recite Hoechst 33342 and BODIPY® which are trade names. The product represented by said trade names can vary with time, thus the claims are rendered indefinite by reference to an object, which is variable.

Claims 1 recites "redundant labeling... with at least two fluorescent labels having the same spectral properties". Claim 7 recites "redundant membrane labeling". Claim 10 recites "cell...labeled redundantly". Claims 20,28 and 37 recite "redundantly labeled". It is unclear what encompasses the claimed redundant labeling as only chemically identical fluorescent moieties would have the same spectral properties. For purposes of examination the redundant fluorescent labels will encompass different antibodies conjugated to identical fluorescent moieties or different chemicals conjugated to identical fluorescent moieties. Thus labeling with any mixture of antibodies or chemicals directed toward different cellular determinants but having identical fluorescent moieties will be considered as redundant labeling.

Claims 1, 7, 10, 35 and 47 are vague and indefinite in the recitation of "up to at least six months" which is a contradiction in terms as "up to" six months means six months or less, but "at least" six months or more.

The recitation of "dye" in claim 8 lacks proper antecedent basis in claim 7.

Claim 16 recites "detectably labeled membrane". Claim 36 recites a "membrane is redundantly labeled". A membrane can acquire a label by means of the direct incorporation of a dye or stain, or by means of antibodies conjugated to fluorescent moieties, wherein said antibodies bind to proteins on the cell membrane. For purpose of examination, a labeled membrane will be confined to the direct incorporation of a stain or dye within the membrane.

The recitation of "the method as claimed in claim 47" lacks proper antecedent basis as claim 47 is drawn to a kit, not a method.

Response:

The relevant inquiry in determining whether a given claim satisfies the requirements of 35 USC § 112, second paragraph is whether the claim sets out and circumscribes a particular area with a reasonable degree of precision and particularity such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (CCPA 1971). Applicants respectively submit, that with respect to the claims in the present aplication, such an inquiry must be answered in the affirmative. The definiteness of claim language may not be analyzed in the abstract, but must be considered in light of the teachings of the prior art and of the particular application disclosure, as it would be interpreted by one having ordinary skill in the art. In re Moore, supra.

Accordingly, applicants' respectfully request reconsideration and withdrawal of this rejection based on the following reasons.

First, the meaning of a "physically and biologically" stable cell is explicitly defined on page 10 para 0101 where the physical stability of a cell defined in reference to an intact cell in suspension. Biological stability is defined as the preservation of antigens present on the cell surface and inside the cell. In addition, a stabilized cell has been defined in terms of several physical properties (i.e. stained, separated, or labeled, see page 7, para 0073). Physical stability is further defined in Example 3 as a function of cell number and storage conditions (page 10, paras 0102 and 0103). Biological stability has been described in the context of preserving the cell structure for analysis (page 7, para 0072). Here, a cell fixed with paraformaldehyde is physically stable for some period of time (i.e. they can be stained, separated, or labeled), but the cellular antigen may not be preserved and would affect any process related to staining, separation, or labeling that would require antigen integrity.

While the applicants acknowledge Hoechst 33342 and BODIPY as trade names, Hoechst 33342 is a very specific dye composition used in labeling double stranded DNA.

BODIPY has been defined in the specifications as a lipophilic form that specifically stains golgi apparatus (page 9, para 0090).

Redundant labeling as used refers to labeling the same or different cellular components with at least two dye compositions, either as free dyes or dyes covalently attached to different antibodies. The dyes have similar, not necessarily identical, spectral characteristics allowing the same excitation source or wavelengths to excite the dyes. The emission profiles are likewise similar and can be analyzed in the same detector or at the same detection wavelengths. The purpose of redundant labeling is to exponentially increase the probability to detect the spiked control cells so as to unequivocally differentiate them from target cells (page 10, para 0094).

For example, a single control label may leave unstained or weakly stain 1 in 1000 control cells. Redundant labeling with a second dye would not only additively enhance the labeling intensity of the already labeled controls cells, but more importantly would reduce the compound probability of non-labeling to about 1 unlabeled cell in 1 million control cells. This high probability of successful control cell labeling is essential for effective discrimination in the highly sensitive assays of this application. This fact is especially relevant since the spiked control cells are phenotypically identical to the endogenous rare target cells detected at extremely low frequencies of about 1 cell in 30mL blood. Labeling the same or two different cellular elements with two structurally and spectrally different fluorophores is a viable option to reduce the probability of misclassification. Page 10, para 0094 describes the labeling conditions. In addition to labeling two different cellular components with spectrally similar fluorophores, labeling two different cellular components with two structurally and spectrally different fluorophores is another type of redundant labeling. Consequently, two cellular components could be labeled with two of the same or different fluorophores.
The applicants respectfully decline to recognize the recitation of "dye" in Claim 8, but acknowledge the lack of proper antecedent basis for the use of "dye in Claim 9. Applicants have corrected this by the foregoing amendment to claim 9.

The recitation of a "detectably labeled membrane" and a "membrane is redundantly labeled" in Claims 16 and 36 refer to the appropriate membrane labeling described in the specifications for direct incorporation of a dye or stain (page 6, para 0058) or by means of antibodies conjugated to fluorescent moieties (page 10, para 0094).

In summary, with respect to this rejection of claims 1-55, in light of the foregoing reasons, it is respectfully submitted that any person skilled in the art having applicants' disclosure and claims before them would be apprised, to a reasonable degree of certainty, as to the exact subject matter encompassed within the claims. Applicants thus assert that nothing more is required under 35 U.S.C. §112, second paragraph. Accordingly, the 35 U.S.C. §112, second paragraph, rejection of claims 1-55 should properly be withdrawn.

CLAIM REJECTIONS – 35 USC § 112, FIRST PARAGRAPH

Detailed Office Action

9. The following is a quotation of the first paragraph of 35 U.S.C. §112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Detailed Office Action

10. Claims 24, 25, 26, 43, 44, 45, 51, 52, and 53 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The specification teaches stabilized cells useful as controls for the detection of rare cells, said stabilized cells carrying determinants, which are the same as said rare cells. Claims 24, 25, 26, 43, 44, 45, 51, 52 and 53 are drawn to CEM T-cell leukemia cells. Raji B-cell leukemia cells and SU-DHL non-Hodgkin's leukemia cells having the labeled determinants of CD4, CD19 and CD20, respectively. However, CD4, CD19, and CD20 are determinants, which are common to hematopoietic cells (Abbas et al., Cellular and

Molecular Immunology, (Textbook), 1991, page 3989). It is not clear how these cell lines carrying the recited labeled determinant would be useful as a control for the detection or rare cells. In the event of screening for residual disease in a patient suffering from T-cell, B-cell or Hodgkin's disease, these cellular determinants would not be unique to the malignancies, therefore one of skill in the art would not be able to use these cells as control cells representing residual cancer cells. One of skill in the art would be subject to undue experimentation in order to discover additional determinants on CEM T-cell leukemia, Raji B-cell leukemia and SU-DHL non-Hodgkin's leukemia cells which would be representative of unique determinants present on the malignant cells of patients suffering from said T-cell, B-cell and non-Hodgkin's leukemias, wherein said determinant would not be present on non-malignant hematopoietic cells. Given the state of the art and the lack of teaching in the specification regarding the above, one of skill in the art would be subject to undue experimentation in order to practice the claimed invention.

Response:

Applicants have, in the foregoing amendment, cancelled the claims that were the subject of this grounds of rejection, without prejudice; therefore, this rejection is now moot and should be withdrawn..

Detailed Office Action

11. Claims 22, 23, 41, 42, 49 and 50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a control cell further comprising a detectable label bound to an intracellular determinant which is an estrogen receptor or an androgen receptor and a method of using said cell in the detection of rare cells, does not reasonably provide enablement for a control cell further comprising a detectably labeled surface determinant which is an estrogen receptor or an androgen receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The art teaches that both the estrogen receptor and the androgen receptor are located in the nucleus (see: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 2001, pages 1606-1607 and pages 1637-1638) within estrogen receptor positive cells, and androgen receptor

positive cells, respectively. Neither the specification nor any art of record teaches a surface determinant which is an estrogen or androgen receptor. One of skill in the art would be subject to undue experimentation without reasonable expectation of success in order find a surface determinant on an MCF-7 cell, which is an estrogen receptor or to find a surface determinant on a LNCaP cell which is an androgen receptor.

Response:

The applicants thank the examiner for correctly pointing out a non-conventional and vaguely defined use of the term "receptor" prefixed by estrogen and androgen in claims 22, 23, 41, 42, 49 and 50. Conventionally, the term "receptors" refer to binders of steroidal hormones that are located in the nucleus according to the cited reference in Goodman and Gilman and not on the cell surface as seemingly implied by the above-cited claims. However in the context of claims 23, 42, and 50, using an androgen "receptor" in conjunction with the surface determinants PSMA and PSA on LNCaP cells, is in conformance to the surface determinants as defined in the specifications. Thus, the androgen "receptor" refers to a type of androgen determinant. The same argument applies to "estrogen receptor" in claims 22, 41, and 49 in reference to MCF-7 breast cancer cells, wherein no specific receptors are recited. As outlined above, the applicants are replacing "receptor" with "determinant" in connection with "estrogenic" or "androgenic" to avoid any confusing use of the term "receptor".

A rejection under 35 U.S.C. §112, first paragraph, based on inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter to which the specification is not enabling. In re Borkowski, 164 U.S.P.Q. 642 (CCPA 1970). Moreover, it is settled law that whenever the adequacy of enablement provided by an applicant's specification is challenged, the PTO has the initial burden of giving reasons, supported by the record as a whole, why the specification is not enabling. In re Armbruster, 185 U.S.P.Q. 152 (CCPA 1975). Indeed, a properly supported rejection showing that the disclosure entails undue experimentation is part of the PTO's initial burden under 35 U.S.C. §112, first paragraph. In re Angstadt, 190 U.S.P.Q. 214 (CCPA 1976).

Accordingly, in view of applicants' responses and the reasoning behind Examiner's doubts expressed regarding the adequacy of enablement provided by the present Specification, it is respectfully asserted that this rejection of claims 22, 23, 24, 25, 26, 41, 42, 43, 44, 45, 49, 50, 51, 52, and 53 based on 35 U.S.C. §112, first paragraph,should be withdrawn with respect to the non-cancelled claims.

CLAIM REJECTIONS – 35 USC § 103

Detailed Office Action

12. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103 (a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 10(e), (f), or (g) prior art under 35 U.S.C. 103(a).

Response:

Applicants respectfully point out that all subject matter of the claims of the present application was/is commonly owned (by Immunivest Corporation) at the time any invention in the application was made.

Detailed Office Action

13. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Detailed Office Action

14. Claims 1,2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis (EP 469,766, reference B1 of the IDS filed Sept. 25, 2001) as evidenced by Leif et al (US 5, 188, 935) in view of Terstappen t al. (WO 95/13540) and Maples (WO 94/16314) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541).

Claim 1 is drawn to a process for producing a stabilized cell, said process comprising: the redundant labeling of said control with at least two florescent labeled having the same spectral properties; contacting said labeled cells with fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable with a fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable for a period up to at least six months. Claim 2 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 4 specifies that the label is an antibody immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 5 is drawn to the labeling of cellular components with DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red and lipophilic BODIPY®. Claim 6 specifies the cellular components of nucleic acids, nuclei, lysosomes, golgi apparatus mitochondria and endoplasmic reticulum.

Claim 10 is drawn to a stabilized cell, wherein said control cell is labeled redundantly with at least two fluorescent labels having the same spectral properties and cellular components and antigenic moieties of said control cell have been stabilized for a period up to at least six months by exposure to fixative. Claim 12 specifies the fixatives of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 14 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 15 specifies that the cellular components of said control cells are labeled with DAPI, Hoechst 33342, acridine orange, rhodamine derivative, neutral red and lipophilic BODIPY.

Claim 28 is drawn in part to a stabilized cell comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being affected by exposure to fixative, wherein said control cells are tumor cells. Claim 29 specifies the fixative of

paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 31 specifies said control cells immunologically specific for an antigen present on said cells, said antibody having a fluorescent conjugate. Claim 32 specifies that the rhodamine derivative, neutral red and lipophilic BODIPY. Claim 33 specifies that the cellular components are nucleic acids, nuclei, lysosomes, golgi apparatus, mitochondria and endoplasmic reticulum.

Davis teaches a method of making a control cell which is fixed by paraformaldehyde, said excess paraformaldehyde being removed after fixing (column 9, lines 20-30). Davis teaches that cells so fixed retain their ability to be tagged with antibodies conjugated to fluorescent markers, stains such as fluorescein, rhodamine, and cyanine dyes and nucleic acid stains (column 5, line 39 to column 6, line 1). Davis et al teach that said control cells may be derived for leukemias, cancers (column 5, lines 16-20, column 8, lines 4-6) and tumor cell lines (column 8, lines 8-11). Davis teaches that the cells may be dried after fixation but before labeling or conversely may be labeled after fixation but prior to drying. Davis does not specifically teach that the fixed cells or the fixed and dried cells would be biologically stabilized for six months, however, the method of paraformaldehyde fixation appears to be identical to the claimed method of fixation, therefore it is reasonable to assume that the fixed cells of Davis would be biologically stable for at least six months.

Davis does not specifically teach the order of the claimed method steps wherein the cells are labeled before being fixed (column 4, lines 4-16). Therefore, it would be reasonable to assume that the control cell of Davis could be labeled before or after fixing cells with glutaraldehyde or glyoxal. Leif et al do not teach the redundant labeling of the fixed cells, the stability of the fixed cells, nor the labeling of tumor cells or tumor cell lines for use as control cells. Davis does not specifically teach the redundant labeling of the cells with at least two fluorescent labels having the same spectral properties.

Maples et al teach differentially labeled reconstituted control cells. Maples et al teach that the use of said cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, lines 8-31). Thus it can be concluded that the control cells of Maples are labeled with the same fluorescent markers as the analyte to

enable the simultaneous analysis of labeled analyte and controls (page 5, line s9-13). Maples et al do not teach control cells exposed to fixative. Gross et al teach the redundant labeling of BT-20 with antibodies, which reacted with the cytokeratins 5, 6, 8 and 18, said antibodies conjugated to the fluorescent moiety PerCP (page 538, second column lines 19-26).

Terstappen et al (WO 95/13540) teach a method of labeling rare cells wherein "one or more monoclonal antibodies, labeled with a first fluorochrome" differentially reacts with all cell populations in the sample (page 5, lines 19-24). Thus it can be concluded that Terstappen et al teach the redundant labeling of analyte cells with a redundant fluorescent moiety. Terstappen et al teach fixed fluorescent cells as control cells (page 6, line 26-29). Terstappen et al do not teach the method of fixing said control cells or the resulting stability of said control cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to redundantly label the control cells taught by Davis with antibodies which immunospecifically bind different target antigens, said antibodies being conjugated to the same fluorescent moiety. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Gross et al and Terstappen et al (WO 95/13540) on the redundant labeling of rare cells, and the teachings of Maples et al on the improvement in the art affected by the use of control cells labeled with the same fluorescent markers as analyte cells.. One of skill in the art would be motivated to redundantly label the control cells because (a) the usefulness of redundantly labeling analyte rare cells has been demonstrated by Gross et al and Terstappen et al, and (b) the teaching of Maples et al directs one of skill in the art to label control cells with the same fluorescent reagents as analyte cells.

Response:

The criterion for determining obviousness under 35 USC § 103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2nd 1929 (Fed. Cir., 1988). Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the

combination. In re Fine, 5 U.S.P.Q. 2nd (Fed. Cir., 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in applicant's disclosure. In re Fritch, 23 U.S.P.Q. 2nd 1780 (Fed. Cir., 1992). Applying these standards to the present case, it is clear that the invention presently claimed is not rendered obvious by the disclosure of Davis and the associated references cited by the examiner.

(a) The applicants contend that the stability of the fixed cells from Davis cannot be assumed to be stable for up to six months. In Davis, cells are fixed with a fixative, such as paraformaldehyde, reduced in the presence of a Schiff's base reducing agent, such as cyanoborohydride, stained with one or more cell markers, dried in the presence of a stablilizing compound for use as a control in cellular analysis (See Davis column 7, lines 25-31). Without further experimentation to determine the long term stability of the cells after reduction, any comparisons with fixed cells not treated with Schiff's base reducing agent would not be appropriate.

(b) The applicants respectfully suggest that Davis does not specifically teach the order of the claimed method steps, but only AFTER fixation (See Davis column 8, lines 45-49). Davis teaches that labeling can be before or after drying, but always after fixation and reduction. Therefore, it cannot be assumed that when combined with Leif, which only confirms antigen-antibody complex preservation after fixation with dialdehyde, antigen sites would remain phenotypically viable after paraformaldehyde fixation.

(c) The applicants further respectfully suggest that light scatter properties are only one component of the characteristics defining control cell use in the simultaneous analysis of the analyte and controls. The analyte and controls are compared through defined cell lines expressing surface markers that are specific to a disease, making them useful candidates for control cells (see page 7, para 0075). Tumor cell lines must first be determined to be suitable as a source of control cells (i.e. they provide surface markers and the capability of accepting additional labels. (See page 8, para 0076).

(d) The applicants suggest that Terstappen teaches differential labeling of beads as an indirect measure of determining cell number based on their peak emission spectra (see Terstappen page 7, lines 15-35). For Terstappen to teach the redundant labeling

of control cells, there would have to be considerable further experimentation. The analyte and controls are compared through defined cell lines expressing surface markers that are specific to a disease, making them useful candidates for control cells (see page 7, para 0075). Tumor cell lines must first be determined to be suitable as a source of control cells (i.e. they provide surface markers and the capability of accepting additional labels. See page 8, para 0076).

(e) The applicants respectfully suggest that one of ordinary skill in the art at the time the claimed invention was made would not be motivated to combine Davis, Leif, Gross, Maples, and Terstappen in a manner that would describe the redundant labeling of control cells.

Davis (EP 468,766) teaches preparation of control cells derived from normal blood, also including leukemia cells, cancer cells and tumor cells lines using conventional labeling methods. This method differs in several critical steps from applicants' invention as follows:

(i) in one preferred embodiment of Davis, the cells are fixed first (see Davis col. 7, lines 25 to 26),

(ii) optionally labeled in a process that does not lock in or stabilize the dye as done in the reverse process of applicants' invention (see Davis col. 7, lines 25-29),

(iii) the cells are then chemically reduced with borohydride to reduce autofluorescence, and then treated with a stabilizer, such as trehalose, that is critical to the stabilization of the lyophilized control cells, but has little stabilizing effect after reconstitution (see Davis col. 8, lines 54 to 57),

(iv) all embodiments recite lyophilization for long-term storage at 4°C (up to 102 days; e.g. see Davis col. 8, lines 28 to 31), and

(v) reconstitution is done prior to use (see Davis col. 12, lines 36 to 45; claim 10).

(vi)

There is no indication of extended stability in the reconstituted form and none is to be expected by the presence of trehalose. No permeabilization is used which accounts for their exclusive use of surface determinants, thus excluding consideration of intracellular determinants and DAPI as a nuclear stain (see Davis col. 11, lines 8 to 21). Hence, control cells prepared by the Davis method would be inherently non-functional in the

assays of applicants' invention due to their lack of intracellular labeling which is absolutely required to identify internal control cells as being phenotypically identical to target cells. The control cells in Davis are intended for flow cytometric analyses of moderate sensitivity. This is appropriate for enumeration of surface labeled target cells like leukocytes that are present at levels >>10/mL specimen. Therefore, they are neither intended nor would they function as internal controls in the highly sensitive assays of applicants' invention.

Similarly, Leif (US 5,188,935) does not teach the preparation of labeled control cells, but rather a method of WBC analysis in whole blood that uses staining of WBC populations with one or more labeled antibodies. For example, Leif refers to RBC lysis with saponin as a "lysing agent... which selectively destabilizes the erythrocyte membrane and accelerates the lysing process" (see Leif col. 4, lines 43 to 48), thus serving a function that is quite dissimilar from applicants'use of saponin in a permeabilization process of an enriched cell population, essentially free of RBC. In Leif, RBC lysis is followed by fixing of the leukocytes with a dialdehyde, in which "Glyoxal is especially preferred in the fixing reagent..." (see Leif col. 4, lines 62 to 64) and "Dialdehydes are preferred over monoaldehydes, such as formaldehyde which have only weak cross-linking action for this purpose" (see Leif col. 3, lines 3 to 5). Hence, Leif teaches away from paraformaldehyde, a polymeric form of the monoaldehyde formaldehyde, as used in applicants'preferred embodiments from Examples 1 and 2. Applicants further contend that neither Davis nor Leif were interested in or prepared control cells that are maximally labeled with a single fluorescent label or redundantly labeled with at least two fluorescent labels as required for internal controls in high sensitivity assays.

The examiner correctly states that Maples (WO 94/16314) teach differently labeled reconstituted control cells having the same size, shape and light scatter characteristics of the analyte (see Maples page 3, lines 8 to 31). Applicants agree with the accuracy of the examiner's statement that "the control cells of Maples are labeled with the same fluorescent markers as the analyte", but in the concluding portion of the sentence, which states "to enable the simultaneous analysis of the labeled analyte and controls," the implication is that this would be appropriate for use as an internal control. This does not reflect the content and intent of the recitation in Maples, page 5, lines 9 to 13. The

objective and sole focus of the control cells in Maples is solely for use as an external control in instrument calibration (see Maples page 5, lines 5 to 14). Here, the objective as stated reads, "The invention describes a method which uses preserved cells to determine light scatter and compensation adjustments for wavelength emissions of multiple fluorescent substances used as labels in multiple color biological analysis. In general, any preserved, labeled cells .... can be used according to the invention." Furthermore, "The selection criteria for any preserved cells ... is that when such cells are reconstituted, washed or other (sic) prepared for use they retain their authentic structural and antigenic characteristics, and that these... are the same as corresponding fresh cells. Such preserved cells, after reconstitution, washing or otherwise being prepared ... are hereby defined as "reconstituted control cells" or simply "control cells." (see Maples page 5, lines 29 to 37). Thus, the emphasis and sole intent is clearly on instrumental calibration with external controls that are preserved, lyophilized and reconstituted before use, thereby suggesting a focused applicability and implying limited stability after reconstitution. The specification of surface labeling with labeled antibodies and the lack of permeabilization of the control cells would affect labeling or staining of intracellular elements, and thus would make the control cells disclosed in Maples non-functional for the intended uses of applicants' invention.

Gross (PNAS 92, 537-541(1995)) does indeed use a variation of redundant labeling of BT-20 breast cancer cells by using a cocktail of fluor-labeled anti-cytokeratin antibodies as recited by the examiner (see Gross page 538, col. 2, lines 19-26). However, this multiple labeling is done after spiking into normal PBMC's as part of a model study in which BT-20 cells are prelabeled enzymatically and covalently with only a single blue fluorescing dye, CMAC (see Gross page 538, col. 1, 2nd paragraph). The singly prelabeled cells serve both as control cells and as target cells in seeding (spike) studies. After fixation and permeabilization, the spiked cells are subsequently labeled with multiple fluors, but recoveries are low, ranging from 10-40% (see Gross page 540, Fig 3 and page 541, Table 1). There are no indications as to stability or storage conditions of CMAC labeled BT-20 cells. Thus, the teaching in Gross differs from applicants' invention, which involves redundant prelabeling of control cells prior to spiking into patient specimens.

Thus, the redundant labeling taught in Gross is not the same as the redundant labeling in the invention claimed in the present application. Gross uses multiple antibodies specific for different sites on the same molecule, but the same fluorochrome whereas applicants' invention uses the same antibody with fluorochromes having the same spectral properties to provide redundant labeling.

Terstappen, in WO95/13540, teaches methods of labeling rare cells wherein "one or more....antibodies, labeled with a first fluorochrome differentially react with all cell populations in the sample (see Terstappen page 5, lines 19 to 24). However, this "redundant labeling" mode differentially labels all cells in the sample as part of the analysis and during analysis, not prior to analysis as is required for spiked internal controls. In Terstappen, the control cell function is performed by adding "a known number of fluorescent beads" (see Terstappen page 5, lines 5 and 6). Furthermore, the prelabeled fixed fluorescent cells (see Terstappen page 6, lines 26 to 29) are part of a subset described as "Beads having these properties may be selected from the group consisting of fixed chicken red blood cells, coumarin beads, liposomes...., fluorescein beads, rhodamine beads, fixed fluorescent cells, ... and other polymeric beads tagged with a fluorescent dye". The list of beads focuses on specific polymeric compositions and fixed non-target cells, and there is no indication that "fixed fluorescent cells" consist of prelabeled fixed target cells, since Terstappen provides no specific means for distinguishing the spiked control cells from the endogenous target cells.

When considering the above arguments, the applicants respectfully submit that it would NOT be obvious to a person of reasonable skill in the art to combine the teachings of Davis as evidenced by Leif and in view of Gross, Maples and Terstappen, and in doing so provide the invention of applicants' claimed subject matter. The present invention focuses on a reproducible, standardized internal control system in the isolation and identification of rare target cells from a sample that also comprises non-target cells. This isolation is based on fluorescent imaging of specific dyes interacting with the membrane and intracellular sites. Antigen labeling with specific antibodies cross-linked to a fluorochrome involve further testing to ascertain the selectivity of the antibody over a range of antigen densities for the target cells. While more than one type of label may be

used to further guarantee that no unlabeled control cells are present, redundant labeling of control cells is not necessarily needed (see page 8, para 0084) for the preferred embodiment. Cell surface markers should be chosen so as to be expressed specifically for the disease of interest, making them useful candidates for control cells (see application page 7 para 0075). In addition, the choice/use of the antibodies should effectively eliminate non-specific binding, to such an extent that tumor cells would be detected at a level of 1 to 10 in a sample containing 100 million leukocytes in a 10 ml blood sample (see page 9, para 0087). This is not taught in Gross/Terstappen in a manner that could be combined with Davis without further experimentation. In fact considering only Davis in light of Gross/Terstappen (without the hindsight of having available the disclosure of the present invention), any determination as to which antibodies would still maintain antigen recognition properties while redundantly labeling membrane and/or intracellular sites would be entirely arbitrary.

One of the more important aspects of the present invention, not mentioned or even suggested in any of the cited references or by any combination of references, is the permeabilization of the control cells prior to fixing. This step is necessary for efficient labeling of control cells in a manner that allows for detection of the labeled control when introduced to the analyte (see page 9, para 0091). Without considerable experimentation, it would be impossible to use labeled fixed cells as an internal control standard. The intracellular antigens targeted by antigen-specific antibodies would not efficiently label the cells, resulting in incomplete labeling and an incorrect assessment of analyte. Accordingly, applicants' submit that combining the references in an obviousness rejection is improper and suggest that this combination would at the very least result in an inoperative combination, without the benefit of hindsight reconstruction based on applicants' own disclosure.

Accordingly since permeabilization is mentioned in applicants' specification and is relevant in the stabilization and labeling of control cells, the appropriate claims have been herein amended to reflect the prior permeabilization of stabilized control cells.

Finally, the applicants agree with the examiner that "One of skill in the art would be motivated to redundantly label the control cells because (a) the usefulness of redundantly labeling analyte rare cells." Consequently, the applicants further contend that with the multiplicity of references needed in combination to meet the present invention AND in light of the above stated need, taken together, is evidence of the non-obviousness of the presently-claimed invention.

Detailed Office Action

15. Claims 1-6, 10, 12-15 and 28-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al as applied to claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32, 33 above, and further in view of Gibson et al (Bone marrow Transplantation, 1988, Vol. 3, pp. 5670576) and Waggoner et al (Human Pathology, 1996, Vol. 27, pp. 494-502) and Haugland (Handbook of Fluorescent Probes and Research Chemicals, 1992, 5th Edition, pp. 235-269).

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al render obvious the embodiments of claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32, and 33 for the reasons set forth above.

Claim 3 specifically embodies the method of claim 1 wherein the fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Claims 13 and 30 specifically embody the control cells of claims 10 and 28, respectively, wherein the fluorescent labels are membrane labels selected from the group consisting of long chain lipophillic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 fluorescein dyes. Although the combination of Davis and Leif et al an Maples et al and Terstappen et al (WO 95/13540) and Gross et al render obvious the labeling of the claimed control cells with fluorescent labels, neither Davis nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al specifically teach the labeling of a cell with the above recited membrane fluorescent labels.

Gibson et al teach the labeling of rare tumor cells with reagents that stain glycolipids (page 567, first column, lines 13-19). Waggoner et al teach the use of diL-C18 as a membrane label to establish the location of the cellular membrane during fluorescence imaging (Table 1, "Membrane location and fluidity").

Dil-C18 is defined as an analog of carboindocyanines as stated on page 25, line 1 of the instant specification.

Haugland teaches the octadecyl ester of fluorescein (page 253, under the heading "Lipophilic Fluorescein Probes"), the octadecyl indocarbocyanines and oxacarbocyanines and analogs thereof, dialkyamnostyrl and octadecyl rhodamine B (pages 260-261), all of which have the property of staining membranes.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the membrane stains taught by Waggoner et al and Haugland for the redundant anti-cytokeratin antibodies as taught by Gross et al in the control cell and method of making said control cell as taught by the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Gibson et al on the labeling of membrane glycolipids in a method of detecting rare tumor cells in bone marrow aspirates and the teachings of Waggoner et al on the usefulness of membrane probes to establish the location and fluidity of the membrane during fluorescence imaging.

Response:

(a) As outlined above (Question 14), the applicants contend that the combination of Davis and Lief and Maples and Terstappen and Gross could not be combined without improper hindsight reconstruction based on applicants' own disclosures, and further experimentation. Thus, the combination would not render obvious the embodiments of the invention as set forth in claims 1,2,4,5,6,10,12,14,15,28,29,31,32 and 33.

(b) Gibson et al does not teach the labeling of rare tumor cells with reagents that stain glycolipids. Gibson et al uses a modified nylon matrix to separate rare cells found in bone marrow (page 574, second column, lines 21-27). Glycoprotein labeling in dual staining of malignant cells was referenced as a procedure that had a controversial

detection limit ($1:10^5$ to $1:10^6$) and was not applicable to the routine study of multiple aspirates (page 567, first column, lines19-3). Glycoprotein labeling using this method would pose the same inherent problems as stated in Gibson. In fact, Gibson et al stated a need in the art for the further enrichment of tumor cells (page 567, second column, lines 3-6), a need not addressed in the combined references, but fulfilled by the present invention (page 5, para 0042). Consequently, combining Gibson et al with the membrane stains taught by Waggoner et al and Haugland would not have the sensitivity needed in assessing the efficiency of rare cell detection without further enrichment (page 1, para 0012). Consequently, the combination of these references produces an inoperative combination.

(c) The applicants are unable to locate page 25 of the prior art.

(d) As outlined above, the applicants respectfully suggest that one of ordinary skill in the art at the time the claimed invention was made would not be motivated to combine Davis, Leif, Gross, Maples, and Terstappen in a manner that would describe the redundant labeling of control cells.

The applicants have considered the additional art disclosed in Gibson, Waggoner, and Haugland, all cited by the examiner. Claim 3 list several long chain lipophilic dyes, and claim 13 and 30 embodies control cells labeled with such dyes none of which were cited by either Davis or Maples or Miles or Terstappen or Gross. Although Waggoner and Haugland teach staining of cell membranes with lipophilic dyes, such art does not address the issue of labeling statistics and the novel concept of redundant labeling that is critical for maximal cell staining. Nor does this art anticipate another novel concept, prelabeled internal control cells, which are phenotypic and thus closely mimic the behavior of target cells. Furthermore, the staining methods advocated by Haugland and others were found to inhibit efficient labeling of cationic lipophilic dyes. Applicants have found washing of the cell cultures with a phosphate-free buffer to be necessary to remove all traces of phosphate, since phosphate was found to precipitate the cationic dyes and thus reduces the staining intensity, uniformity and the extent of labeling of both suspended or adherent cells. This phenomenon may have escaped detection since membrane staining is normally sufficiently bright for most conventional applications. But maximal and uniform

staining as a result of applicants' modified staining protocol is believed to be a critical factor in applicants' applications. A closer review of Gibson shows labeling of the nuclei of 2 cell lines, SK-NH-SH and JR1, not of cell membranes as recited, with the intercalating DNA dye, Hoechst 33342. None of the cited art discloses nor anticipates the elements previously discussed and thus do not render them obvious. Gibson proposes a model for separation of rare cells from bone marrow. This would require optimizing the conditions for labeling of membrane glycolipids, a need that could only be addressed through extensive experimentation and not obvious from the combination of references alone (page 574, second column, lines 39-51).

Detailed Office Action

16. Claims 1-10 and 12-16, 18-21, 23 and 28-33 and 35-40, 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al. and Waggoner et al and Haugland as applied to claims 1-6, 10, 12-15 and 28-33 above, and further in view of Racila et al (PNAS, 19998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) as evidenced by the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96). The combination of Davis and Leif et al. and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland render obvious the embodiments of claims 1-6, 10, 12-15 and 28-33 for the reasons set forth above.

Claim 7 is drawn to a process for producing a stabilized control cell comprising: the redundant labeling of said control cell with at least two florescent labels having the same spectral properties; contacting said labeled cells with a fixative; removing excess fixative to promote long-term storage, said control cells being biologically stable for a period up to at least six months, wherein said control cell expresses epithelial cell adhesion molecule on its surface and also expresses cytokeratin intracellularly. Claim 8 specifically embodies the process of claim 7 wherein cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 9 specifically embodies the process of claim 7 wherein the fluorescent label is a membrane dye selected from the group consisting of

long chain lipophillic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes.

Claim 16 is drawn to a stabilized cell having determinants in common with rare cells, said stabilized cell comprising a detectably labeled membrane and stabilized cellular components and antigenic moieties due to exposure to fixative, wherein said control cell is a tumor cell expressing EpCAM on its surface and intracellular cytokeratin. Claim 18 specifically embodies the cell of claim 16 wherein said fixative is selected from the group consisting of paraformaldehyde, formaldehyde and glutaraldehyde and glyoxal. Claim 19 specifically embodies the cell of claim 16 wherein said membrane label is selected from the group consisting of long chain lipophillic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes. Claim 20 specifically embodies the cell of claim 16 wherein said membrane is redundantly labeled with at least two fluorescent moieties having the same spectral properties. Claim 21 specifically embodies the control cell of claim 16, wherein said cell is a SKBR3 breast cancer cell further comprising a second detectably labeled surface determined selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu. Claim 23 comprises the control cell of claim 16 wherein said cell is a LNCaP prostate cancer cell further comprising a detectably labeled determinant selected from the group consisting of PSMA, PSA and the androgen receptor.

Claim 35 is drawn to a method of detecting an enumerating rare cells in mixed cell population, the presence of said cells being indicative of severity of a disease state comprising:

(a) obtaining a blood sample suspected of containing said rare cells

(b) preparing an immunomagnetic sample wherein said biological specimen is mixed with magnetic particles coupled to a ligand which reacts specifically with a determinant of the rare cells.

(c) contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells, and

(d) analyzing the labeled rare cells to determine the presence and number of any rare cells, wherein the greater the number of rare cells present in said sample, the greater the severity of the disease, wherein the improvement comprises the

addition of a stabilized cell for use as an internal control in said method, said control cell having determinants in common with said rare cells, and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for a period up to six months by exposure to fixative.

Claim 36 specifically embodies the method of claim 35 wherein said rare cell is a cancer cell and said disease is cancer. Claim 37 specifically embodies the method of claim 35 wherein said membrane is redundantly labeled with at least two fluorescent labels having the same spectral properties. Claim 38 specifically embodies the method of claim 35 wherein said membrane label is selected from the group consisting of long chain lipophillic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and C18 fluorescein dyes. Claim 39 specifically embodies the method of claim 35 wherein said ligand is an anti-EpCAM, said reagent labels intracellular cytokeratin, said EpCAM and Cytokeratin being present in both rare cell and control cell. Claim 40 specifically embodies the method of claim 35 wherein the control cell is an SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu. Claim 42 specifically embodies the method of claim 39 wherein the control cell is an LNCaP prostate cancer cell further comprising a second detectably labeled determinant selected from the group consisting of PSMA, PSA and androgen receptor.

The combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland render obvious the process of producing a control cell comprising a redundantly labeled membrane, the membrane stains of long chain lipophillic: carbocyanines, indocarbocyanines, indodicarbocyanines and analogs thereof, aminostyrl dyes, C18 rhodamine B, and the control cell produced thereby. The combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland do not teach a method of producing a stabilized control cell expressing epithelial cell adhesion molecule on its surface, a stabilized tumor cell expressing EpCAM on its surface, a stabilized SKBR3 breast cell expressing EpCAM on its surface and further comprising a detectably labeled surface determinant selected from

the group consisting of mammoglogulin, human milk-fat globulin and Her-2/neu, or a stabilized LNCaP prostate cancer cell expressing EpCAM on its surface and further comprising a detectably labeled determinant selected from the group consisting of PSMA, PSA and androgen receptor or an immunomagnetic separation of cancer cells from normal cells wherein the detection of said cancer cells is correlated with the severity of the disease and wherein the method is improved by the addition of control cells, said control cells having determinants in common with rare cells. However, it is noted that the combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al do render obvious the control cell comprising the determinant of intracellular cytokeratin as taught by Gross et al (page 538, second column, second paragraph).

Racila et al teach the detection of rare prostate cancer cell and rare breast cancer cells in the blood of patients. Racila et al teach that the greater the number of said rare cells the greater the severity of the disease (page 4592, first column, line 19 to second column, line 2 and Figure 4). Racila et al teach the use of SKBR3 breast cancer cells and LNCaP prostate cancer cells as standards to evaluate the reagents used in the immunocytochemical detection and to determine the sensitivity of the assay (page 4589, second column, under the heading "Cell Lines"). Racila et al teach an immunomagnetic separation of rare prostate cancer cells or rare breast cancer cells from biological specimens, said assay using the ligand anti-EpCam (page 4589, beginning in second column, under the heading of "Sample Preparation for Flow Cytometric Analysis"). Racila et al further teach the labeling of said separated cells with the anti-cytokeratin CAM5.2 antibody (page 4590, first column, lines 6-8), and the further labeling of said separated prostate cells with PSA and the redundant labeling of cytokeratins by means of monoclonal antibodies CK, 5D3, and LP34 (page 4590, first column, lines 24-30). Racila et al do not teach the SKBR3 breast cell further comprising a detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin and Her-2/neu. Racila et al do not teach a method of producing SKBR3 or LNCaP as stabilized control cells comprising redundantly labeled membranes, or the control cells produced thereby, or an improvement to the immunomagnetic separation comprising the addition of said control cells as internal controls.

Terstappen et al (Interantional Journal of Oncology, 2000, Vol. 17, pp. 573-578) teach immunomagnetic separation of rare breast cancer cells from biological specimens, said assay using the ligand anti-EpCam (page 573, second column under the heading "Sample preparation") and the further labeling of separated cells with the anti-cytokeratin antibody CAM5.2 (page 574, first column, lines 2-6). Terstappen et al teach a correlation between the number of said rare cells the severity of the disease (Figure 2). Terstappen et al suggest a further assessment of the isolated rare breast cancer cells comprising the detection of mammoglobin, breast mucin and her-2 (page 577, second column, last eight lines). The abstract of Xing et al teaches that human milk-fat globulin is a type of mammary mucin that is incrased in breast cancer.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the specific cells of SKBR3 or LNCaP as stabilized control cells comprising redundantly labeled membrane for the general tumor cell line control dells taught by the combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland: and to use said stabilized cells as an improvement to an immunomagnetic separation comprising the addition of said cells as internal controls. It would also be obvious to produce a stabilized SKBR3 control cell further comprising a detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin and Her-2/neu.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Racila et al and Terstappen et al on the use of SKBR3 cells and LNCaP cells as standards in the evaluation of immunocytochemical detection and the teachings of Maples et al on the improvement afforded to methods for detecting rare cells by the incorporation of biological cells as control cells and the teachings of Terstappen et al (WO 95/13540) on the use of fixed fluorescent cells as control cells in the enumeration of rare cells by flow cytometry (page 6, lines 26-29 and page 5, line 19 to page 6, line 4). One of skill in the art would also be motivated by the teachings of Terstappen et al (International Journal of Oncology, 2000) and Xing et al to further label said control cells of the determinants of milk-fat globulin, mammoglobin and Her-2, as these wee suggested by Terstappen

(2000) to further study the correlation between said detection of said determinants in blood and the presence of metastatic breast cancer (page 577, last ten lines).

Response:

(a) As stated above, the applicants contend that the combination of Davis and Leif and Maples and Terstappen and Gross could not be combined without hindsight and further experimentation. Thus, the combination would not render obvious the embodiments of Claims 1-10 and 12-16, 18-21, 23 and 28-33 and 35-40, 42.

(b) Applicants note that the prior art reference (page 538, second column, second para) does not refer to the stated issues. Applicants further note that the combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al do not make the control cell comprising the determinant of intracellular cytokeratin as taught by Gross et al (page 538, second column, second para) obvious. In addition to the above stated reasons, it would be necessary to make modifications, not taught in the prior art, in order to combine Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland. Gross et al uses flow cytometry in rare cell detection. The redundant immunofluorescence markers are used to enhance the detection of the cytokeratins. Detection of cytokeratins in the present invention would involve modification of the instrument settings such as the setting for the threshold on the forward light scatter (page 13, para 0125). This permits the elimination of non-desired events including ferrofluid particles and residual fluorescently labeled antibodies based on size.

(c) The combination of references would not be obvious (Question 14 and 15). Combining Terstappen (2000) and Xing et al could only be made after extensive experimentation. Terstappen suggests the use of a cancer cell detection assay to determine the origin of said tumor cell. Xing et al suggests the presence of mammary mucins in breast cancer patient sera. Labeling control cells with antibodies to said determinants could only occur after complete analysis of antigen density (page 8, para 0085) and aspects of instrument settings (page 13, para 0125). The examiner referes to the element of fixation and its effects on capture via the EpCAM marker and of detectably labeling surface determinants, specifically mammoglobin, human milk-fat globulin and Her-2/neu on SKBR3 cells and PSMA, PSA and androgen receptors on LNCaP. The method of fixation and particularly the absence of residual fixative were

found to be critical to long-term stability of up to 270 days, as judged by physical stability or remaining cell number (Ex. 3; pages 27 and 28; Table 1a) and by biological or functional stability (Ex. 3; pages 28 and 29; Table 1b). Again, these issues would only be obvious with the benefit of hindsight after extensive experimentation.

(d) Racila adds the elements of rare prostate cancer and breast cancer cell detection in patient samples. In both cases spiked unlabeled cancer cells were spiked as "standards" to assess reagents performance as judged by recoveries. Racila used no prelabeled internal control cells. Consequently, it would not be obvious to combine with other references. Terstappen teaches immunomagnetic selection using EpCAM for capture and a labeled anti-cytokeratin antibody with suggested detection of mammoglobin, breast mucin and Her-2/neu. Xing add human milk fat globulin to this list. Neither citation involves prelabeled control cells. Hence, the art as taught in Davis, Leif, Terstappen (WO95 and 2000), Maples, Gross, Gibson, Waggoner, Haugland, Racila and Xing, viewed individually or in combination, would not support an obvious recognition of the essential elements of this invention.

Detailed Office Action

17. Claims 1-21, 23, 28-40 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al as applied to claims 1-10 and 12-16, 18-21, 23, 28-33 and 35-40, 42 above, and further in view of Young et al (US 5,529,933).

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (Interantional Journal of Oncology, 2000, Vol. 17, pp. 5730578) and Xing et al render obvious the embodiments of claims 1-10 and 12-16, 18-21, 23, 28-33 and 35-40, 42 for the reasons set forth above.

Claim 11 specifically embodies the control cell of claim 10, said control cell suspended in a buoyant density medium.

Claim 17 specifically embodies the control cell of claim 16, said control cell suspended in a buoyant density medium.

Claim 34 specifically embodies the stabilized cell of claim 28, said control cell suspended in a buoyant density medium.

Although the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (Interantional Journal of Oncology, 2000, Vol. 17, pp. 57-578) and Xing et al render obvious the stabilized fixed cells of the instant invention, neither Davis nor Leif et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) teach the stabilized, control cells suspended in a buoyant density medium.

Young et al teach stabilized red blood cells fixed with organic aldehydes such as formaldehyde or glutaraldehyde (column 10, lines 11-27) for use as a control in hematological analyses (column 11, lines 61-67). Young et al teach that said fixed red blood cells can be maintained for up to six months in a preferred formulation of a suspension medium (column 14, line 61 to column 15, line 39). Young et al do not teach cells with fluorescent labels for use in detecting rare cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to suspend the stabilized control cells as rendered obvious by the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Jour'nal of Oncology, 2000, Vol. 17, pp. 573-578) in buoyant density medium. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Young et al on the long-term stability of aldehyde fixed cells stabilizing medium.

Response:

As stated above, Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al. and Waggoner et al and Haugland as applied to claims 1-6, 10, 12-15 and 28-33 above, and further in view of Racila et al (PNAS, 19998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) as evidenced by the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96) could possibly be combined, but only after extensive experimentation and testing. By themselves, the references do not teach exactly how to combine their teachings into the present invention. It is only in hindsight that a way to combine the references can be seen.

As correctly noted by examiner, Young (U.S. 5,529,933) teaches stabilization of RBC with organic aldehydes including formaldehyde (col. 10, lines 11to 27) for use as controls in calibrating hematology analyzers (col. 11, lines 61 to 67) and that such fixed RBC are stable for up to 6 months in a preferred suspension medium (col. 14, line 61 to col. 15, line 39). The applicants would like to point out that Young does not teach fluorescent labeling nor does he teach detection or preservation of cellular determinants on such stabilized controls for the stated six-month period. The primary utility of these stabilized cells are use as cell analogs that simulate or mimic the morphologic characteristics of blood cell populations, e.g. they constitute "analogs of specific size and volume increments and light scatter characteristics for use as a quality control." (col. 6, lines 61 to 63). Without considerable further experimentation, applicants contend that extrapolation of the preferred formulation for the suspension medium (col. 15, lines 12 to 38, "for conferring long term stability for red blood cells and analogs") could not preserve both determinants and fluorescent labels on internal control cells for extended time periods. The preferred suspension media may have included a "buoyant density medium" but the expression "buoyant" is not recited in Young nor is there any indication that this desirable characteristic was observed, utilized or appreciated in Young. Thus,

applicants respectfully submit that the addition of Young to the combination of prior art does not support the obviousness assessment by the examiner.

Detailed Office Action:

18. Claims 1-23, 28-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95 pp. 4589-4594) and Terstappen et al (International Japplicants'nal of Oncology, 2000, Vol. 17, pp 57-578) and Xing et al and Young et al as applied to claims 1-21, 23, 28-40 and 42 above, and further in view of Rao et al (Cancer, 1980, Vol. 46, pp. 2902-2906).

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Japplicants'nal of Oncology, 2000, Vol. 17 pp. 573-578) and Xing et al and Young et al render obvious the embodiments of claims 1-21, 23, 28-40 and 42 for the reasons set forth above.

Claim 22 specifically embodies the control cell of claim 16, wherein said control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor.

Claim 41 specifically embodies the method for claim 39, wherein the control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor.

Please note the limitation of "surface' determinant has been omitted from the claims for the reasons set for the in the rejection under 35 U.S.C. 112, first paragraph, above.

Although the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Jour'nal of Oncology, 2000, Vol. 17, pp. 573-578) render obvious the methods and control cells comprising SKBR3 breast cancer cells, neither Davis nor Leif et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17,

pp. 573-578) nor Xing et al nor Young et al teach a control cell which is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor or a method of detecting and enumerating rare cells comprising the use of said control cell.

Rao et al teach a MCF-7 control cell comprising a detectably labeled estrogen receptor 2903, first column, under the heading "Staining with Fluorescein Conjugate" and the use of said control cells in the detection of breast cancer (page 2905, first column, lines 18-23 and page 2904, second column, under the heading "17FE Uptake in Human Breast Cancer). Rao et al suggest the method of flow cytometry as a more precise method of quantitating the fluorescence intensity observed in tumor cells stained for the estrogen receptor. Rao et al do not teach a fixed MCF-7 cell or the use of said fixed control cell in an assay for rare cells.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the MCF-7 control cell for the fixed SKBR3 cell.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Rao et al on the presence of estrogen receptors in both MCF-7 cells and human breast cancer, and the use of MCF-7 cells as control cell in the detection of estrogen receptors in human cancers.

Response:

As stated above, the applicants argue that Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al. and Waggoner et al and Haugland and further in view of Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96) could not be combined without the benefit of hindsight from extensive experimentation.

The examiner contends that it would be an obvious step to replace MCF-7 cancer cells bearing detectably labeled estrogen determinants in flow cytometry as disclosed by Rao with detectably labeled SKBR-3 control cells. Rao used prelabeled and unfixed cells for external calibration, not for control purposes, i.e. "as a more precise method of

quantitating the fluorescence intensity observed in tumor cells stained for estrogen receptor." The prelabeled internal controls, used by applicants, are for two purposes: monitoring assay performance and cell recovery, both of which were not addressed by Rao. Furthermore, the unfixed labeled cells would be stable for only a few days. There is no indication that these control cells are redundantly labeled and functional equivalent in the applications of applicants' invention. Taken together, these points support applicants' argument that the combination of references could not be completed without further experimentation. Accordingly, for these reasons applicants' believe withdrawal of this grounds of rejection is proper.

Detailed Office Action

19. Claims 1-23, 28-42 and 47-50, rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al as applied to claims 1-23, 28-42 above, and further in view of Terstappen et al (WO 99/41613).

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al render obvious the embodiments of claims 1-23, 28-42 for the reasons set forth above.

Claim 28 is further drawn to a stabilized cell comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being affected by exposure to fixative, wherein said control cells are selected from the group consisting of tumor cells, bacterially infected cells, virally infected cells, myocardial cells, circulating endothelial cells, and fetal cells in maternal circulation.

Claim 47 is drawn to an improved kit for the screening of a patient for the presence of circulating tumor cells comprising:

(a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material and anti-EpCAM coupled directly or indirectly to said base coating material;

(b) at least one antibody having a binding specificity for a cancer cell determinant;

(c) cell specific dye for excluding sample components for other than said tumor cells; wherein the improvement comprises the addition of a container comprising stabilized cells for use in an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for up to at least six months by exposure to fixative, said stabilized control cells being suspended in buoyant density medium.

Claim 48 specifically embodies the method of claim 47, wherein said cell is a SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu.

Claim 49 specifically embodies the method of claim 47, wherein said control cell is a MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor.

Claim 50 specifically embodies the method of claim 47, wherein said cell is a LNCaP prostate cancer cell further comprising a detectably labeled determinant selected from the group consisting of PSMA, PSA and androgen receptor.

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al renders obvious the stabilized control cells, wherein said cells are SKBR3 breast cancer cell further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk-fat globulin, and Her-2/neu; MCF-7 breast cancer cell further comprising a detectably labeled determinant which is an estrogen receptor; and LNCaP prostate cancer cell further comprising a detectably

labeled surface determinant selected from the group consisting of PSA and the use of said control cells in an method for the detection and enumeration of rare tumor cells using an immunomagnetic separation for the reasons set forth above.

Davis further teaches a control slide kit for image analysis comprising cells which have been fixed with paraformaldehyde, reduced with a Schiff's base after fixation and then dried onto a slide or into a capillary tube with a protein, membrane stabilizing compounds (column 5, lines 23-28). Davis does not specifically teach tumor cells having the Ep-CAM determinant, or the enumeration and isolation of rare cells in circulation.

Maples et al teach differentially labeled reconstituted control cells. Maples et al teach the use of said cells represents an improvement in the art over the use of fluorescent beads as said cells have the same size shape and light scatter characteristics of the analyte (page 3, line 8-31). Thus it can be concluded that the control cells of Maples are labeled with the same fluorescent markers as the analyte to enable the simultaneous analysis of labeled analyte and controls (page 5, lines 9-13). Maples et al do not teach control cells exposed to fixative.

Terstappen et al (WO 95/13540) teach fixed fluorescent cells as control cells (page 6, lines 26-29). Terstappen et al do not teach the method of fixing said control cells or the resulting stability of said control cells.

Young et al teach stabilized red blood cells fixed with organic aldehydes such as formaldehyde or glutaraldehyde (column 10, lines 11-27) for use as a control in hematological analysis (column 11, lines 61-67). Young et al teach that said fixed red blood cells can be maintained for up to six months in a preferred formulation of a suspension medium (column 14, line 61 to column 15, line 39). Young et al do not teach cells with fluorescent labels for use in detecting rare cells. However, Leif et al teach the maintenance of antibody-cell complexes through the aldehyde fixing process.

Terstappen et al (WO 99/41613) teach a kit for the screening of a patient for the presence of circulating tumor cells comprising:

(a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material and anti-EpCAM coupled directly or indirectly to said base coating material;

(b) at least one antibody having a binding specificity for a cancer cell determinant;

(c) cell specific dye for excluding sample components for other than said tumor cells claims 48-62 of WO 99/41613).

Terstappen et al (WO 99/41613) further tech the claimed immunomagnetic method for the detection of circulating rare cells selected from the group consisting of endothelial cells, fetal cells in circulation, bacterial cells, myocardial cells and virally infected cells (claim 6).

Neither Davis nor Leif et al nor Maples et al nor Terstappen et al (WO 95/13540) nor Gross et al nor Gibson et al nor Waggoner et al nor Haugland nor Racila et al nor Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) nor Xing et al nor Young et al and Rao et al teach an improved kit for the screening of a patient for the presence of circulating tumor cells comprising: a container comprising stabilized cells for use in an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cells have been stabilized for up to at least six months by exposure to fixative, said stabilized control cells being suspended in buoyant density medium, wherein the control cells are SKBR3, MCF-7 or LNCaP.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to

(a) use the control cells rendered obvious by the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al as an improvement to the kit for detecting circulating rare cells in patient; and

(b) to make stabilized control cells comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same

spectral properties, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being affected by exposure to fixative, wherein said control cells are , in addition to the tumor cells rendered obvious by the combination of the prior art references stated in this section, are selected from the group consisting of bacterially infected cells, virally infected cells, myocardial cells, circulating endothelial cells, and fetal cells in maternal circulation.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Davis on a kit comprising paraformaldehyde fixed cells for use as a control slide in image analysis, and the teachings of Terstappen et al (WO 95/13450) on the use of fixed fluorescent cells as control cells, and the teachings of Maples et al on the improvements afforded by the use of biological cells as control cells (versus fluorescent beads) and the teachings of Young et al on the stability of a cell fixed with aldehydes and stored in a suspension medium; and the teachings of Terstappen et al (WO 99/41613) on an immunomagnetic method for the detection of circulating rare cells selected from the group consisting of endothelial cells, fetal cells in circulation, bacterial cells, myocardial cells and virally infected cells.

Response:

applicants wish to point out that the examiner's reference to Terstappen et al (WO 95/13450) is not to a known reference. For purposes of this response, applicants will consider the reference Terstappen et al (WO 95/13540).

(a). As stated above, claims 1-23, 28-42 related to the combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al. and Waggoner et al and Haugland and further in view of Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96) and Young et al and Rao et al can not be combined for use as a control cell and further can not be combined as an improvement to a kit for detecting circulating rare cells in patients without extensive experimentation and hindsight from the present application.

Davis does not teach a control slide kit for image analysis comprising cells, which have been fixed, reduced and dried, but rather a method for preparing cells which retain their light scatter properties and their ability to be tagged with cell markers (column 5, lines 23-28).

(b) The production of a stabilized control cell comprising a redundantly labeled membrane, having the same spectral properties, would not be obvious to a person of ordinary skill in the art, especially with the stabilized control cell further having stabilized cellular components and antigenic moieties. As stated above, applicants argue that the combination of Davis and Leif et al and Terstappen et al and Maples et al and Gross et al and Gibson et al. and Waggoner et al and Haugland and further in view of Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and the abstract of Xing et al (Cancer Research, 1990, Vol. 50, pp. 89-96) and Young et al and Rao et al can not be asserted without an extensive appreciation for the selected antigens and cell line in the context of their use as a stabilized control cell according to the present application.

Detailed Office Action

20. Claims 1-23, 27-42 and 46-50 and 54 are rejected under 35 U.S.C. 103(a) as being unpatentable over Davis and Terstappen et al and Leif et al and Maples et al and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al (PNAS, 1998, Vol. 95, pp. 4589-4594) and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) as applied to claims 1-23, 28-42 and 47-50 above, and further in view Shih (Journal of Pathology, 1999, Vol. 189, pp. 4-11) and Shih et al (Cancer Research, 1994, Vol. 54, pp. 2514-2520) and the abstract of Silverstein et al (Journal of Biological Chemistry, 1992, Vol. 267, pp. 16607-16612).

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO

99/41613) render obvious the embodiments of claims 1-23, 28-42 and 47-50 for the reasons set forth above.

Claim 27 specifically embodies the control cell of claim 16, wherein said control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

Claim 46 specifically embodies the method of claim 39, wherein the control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD146 molecule.

Claim 54 specifically embodies the kit of claim 47, wherein the control cell is a C32 melanoma cell further comprising a second detectably labeled surface determinant which is a CD 146 molecule.

The combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573- 578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) render obvious the claimed control cells, methods and kits of the instant claims 27, 46, and 54 with the exception of the C32 melanoma cancer cell detectably labeled on the CD 146 molecule.

Terstappen et al (WO 99/41613) teach that melanoma can be detected using the immunomagnetic compositions, kits and methods disclosed therein (page 75, lines 8-10 and 17). Terstappen et al do not teach C32 control cells comprising a detectable label on the CD 146 molecule.

The Shih (1999) teaches that the detection of CD146 is useful in the diagnosis of melanoma (abstract). Shih further teaches that a synonym for the CD146 antigen is the A32 antigen (page 4, second column, lines 1-6).

Shih et al (1994) teach that the A32 antigen is expressed on the cell surface of most melanomas and on melanoma cell lines (abstract and Figure 7 on page 2519).

The abstract of Silverstein et al teaches that C32 cells are wild-type melanoma cells. It would be reasonable to conclude that C32 cells expressed the CD146/A32 antigen.

Neither the abstract of Shih or Silverstein et al teach fixed stabilized C32 cells comprising a detectably labeled CD146 molecule.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute C32 cells having a detectably labeled cell surface determinant which is a CD146 molecule for the general tumor cells taught by Davis.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of the abstract of Silverstein on C32 cells being representative of melanoma and the teachings of Shih et al on the CD146/A32 molecule as being diagnostic for melanoma and the presence of the CD146/A32 antigen on melanoma cell lines.

Response:

As stated above, the combination of Davis and Leif et al and Maples et al and Terstappen et al (WO 95/13540) and Gross et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstappen et al (International Journal of Oncology, 2000, Vol. 17, pp. 573-578) and Xing et al and Young et al and Rao et al and Terstappen et al (WO 99/41613) is not prooper, for the reasons set forth, without the benefit of hindsight. So, while claim 28 refers to stabilized cells, which are redundantly labeled in the membrane also bearing determinants stabilized by fixation, claim 47 references a kit containing stabilized redundantly labeled internal controls, claims 28, 49, and 50 refer to specific types of cancer cells used for preparing such control cells 27, 46, and 54 embody C32 melanoma cells bearing a CD146 determinant as disclosed in Shih and Silverstein. For each of these claims, however, the recitation of art, teaching only about the diverse cell types or determinants, comprises only a limited aspect of this invention, which must be evaluated and judged in its totality that transcends mere additive contributions from numerous investigators and not enabling the full scope of the invention. Among the plethora of tissue types (and neoplasms) that express CD146, expression of CD146 based on immunostaining, the staining intensity varies within a tumor and between tumor types (Shih, page 6, column 2, para 2). Immunoreactivity for any given cell type is variable and may depend on the state of differentiation (Shih, page 6, column 2, para 3). Accordingly, applicants respectfully assert that combining Shih and Silverstein in the

context of fixed stabilized C32 cells for use with a detectably labeled CD146 would not be obvious to one of ordinary skill in the art without further extensive experimentation.

Detailed Office Action

21. All claims are rejected.

22.

Response:

In summary, as has been shown above, the rejection of all of applicants' claims based on alleged obviousness in the recited combined art does not reflect the creativity and experimentation needed to conceive and successfully develop the desirable properties of the detectably and redundantly labeled control cells of this invention, which make them uniquely useful for monitoring both systematic and random errors. Some of the recited investigators addressed systematic errors by means of external control cells, but no one used optimally labeled internal control cells capable of monitoring both factors, which are essential in commercially viable diagnostic assay components or kits for detection of rare target cells.

Moreover, applicants believe that the 35 USC § 103 rejections set forth in the July 18, 2002 Official Action can only be maintained by being premised on a hindsight reconstruction of the claimed invention. The examiner appears to have used Applicants' disclosure as a guide for combining prior art disclosures that would otherwise require extensive experimentation in an effort to make out a case for obviousness. Under 35 USC § 103, a determination must be made as to whether the claimed subject matter AS A WHOLE would have been obvious at the time the invention was made, based on the prior art disclosures, and without the benefit of applicants' disclosure.

Viewed objectively and without the benefit of applicants' disclosure as a guide for selection, none of the cited references teach, nor is there any suggestion in any of them or in any combination of them, to use a pre-labeled, stabilized cell as an internal functional control in cell selection and analysis.

In view of the amendments and foregoing discussion and arguments, it is respectfully urged that the rejections set forth in the July 18, 2002 Official Action should be withdrawn and that this application be passed to issue. In the event the examiner has any comments or questions, the examiner is invited to telephone applicants' undersigned representative at the number below.

Respectfully submitted,

By Joseph F. Aceto

Joseph F. Aceto, Ph.D., J.D.
PTO Registration No. 50,701
Telephone No. 215-830-0777, x237

Figure 1: Flow cytometric analysis of control cells

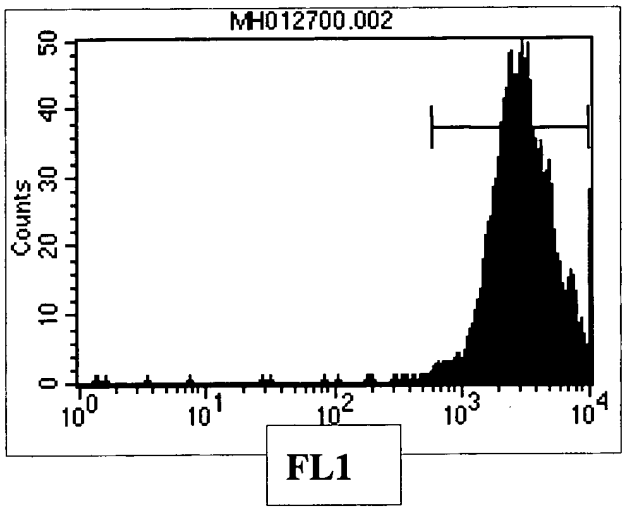


Figure 1a

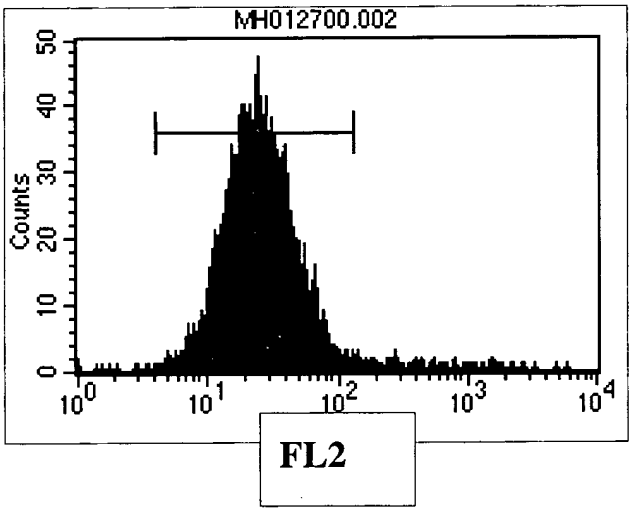


Figure 1b

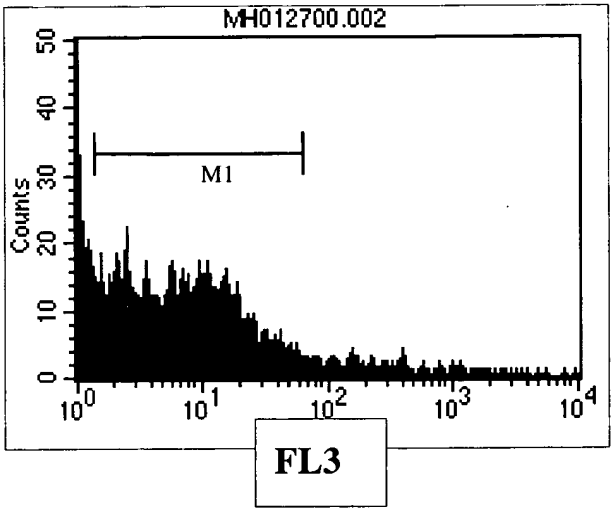


Figure 1c

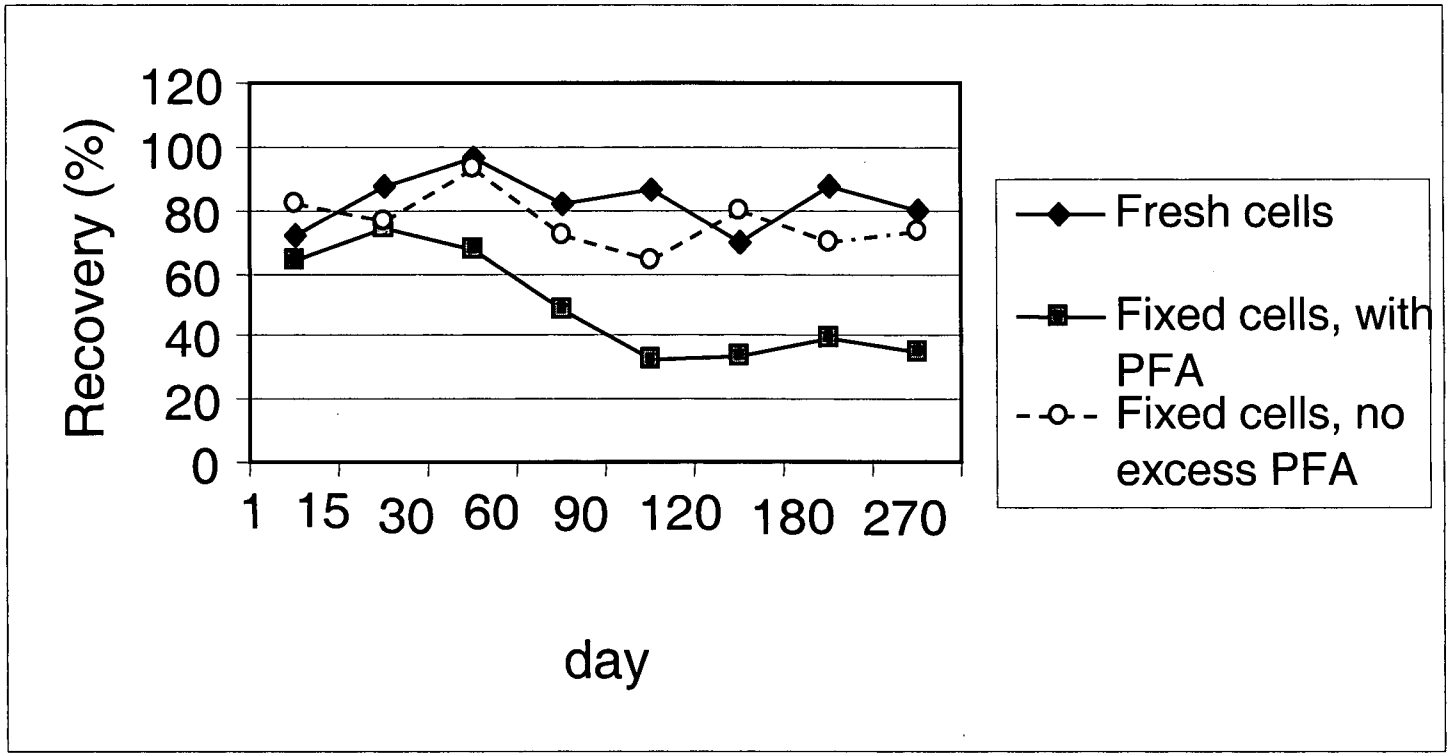


Figure 2

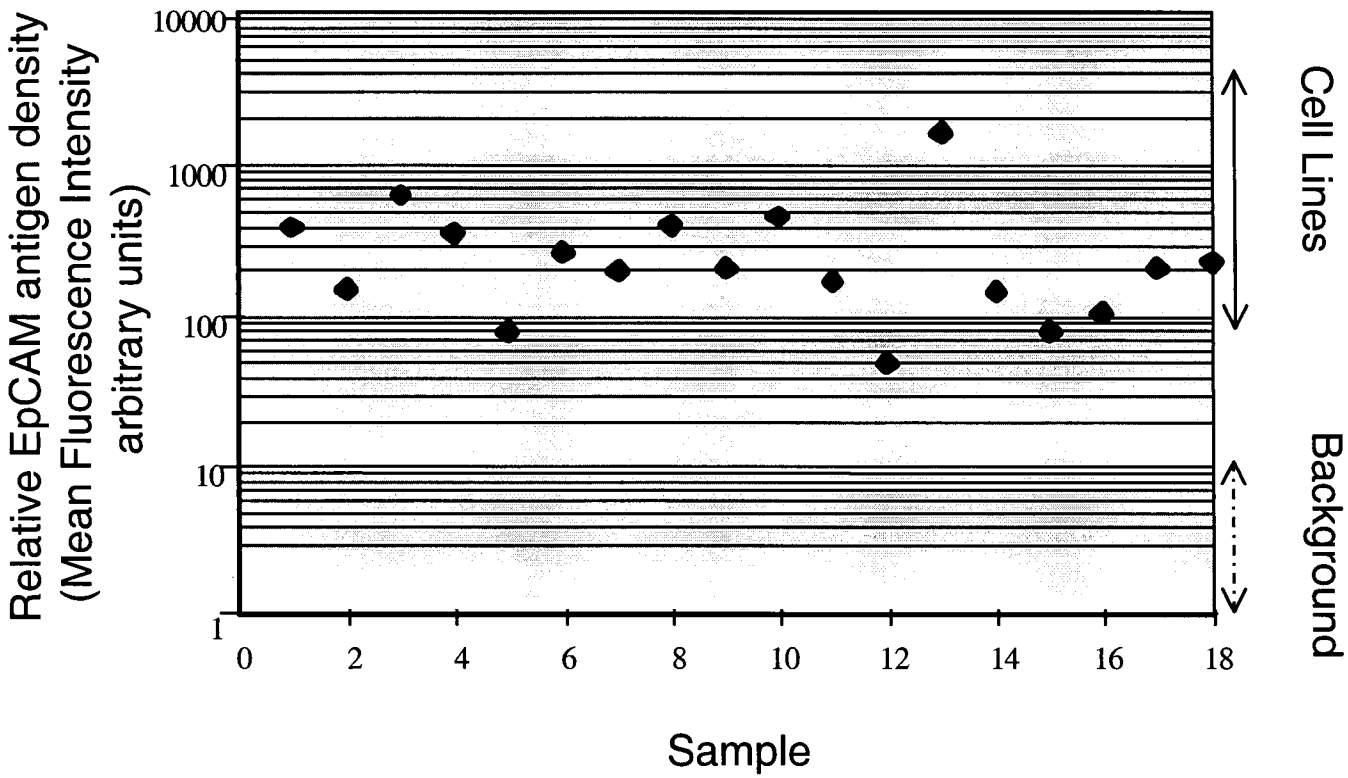


Figure 3

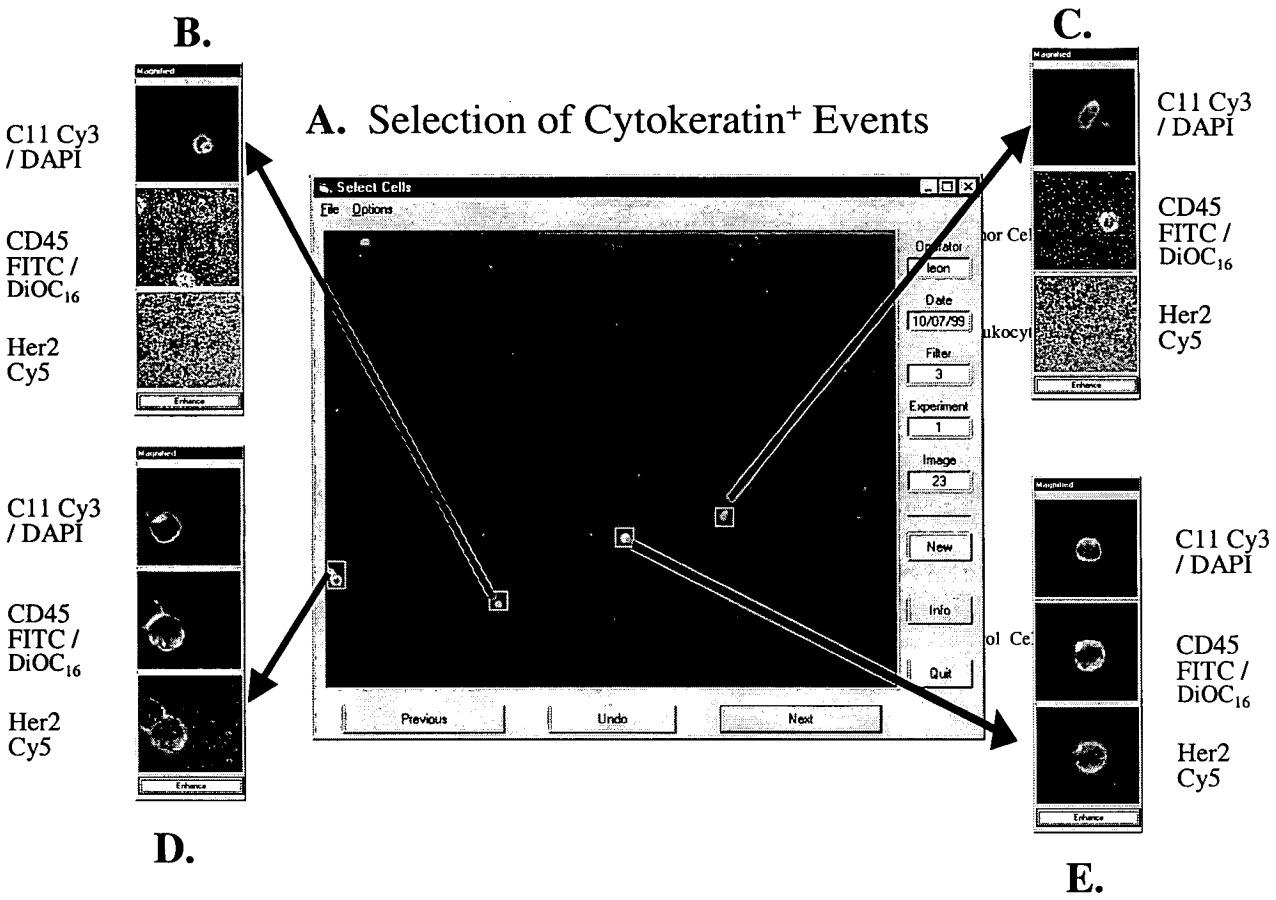
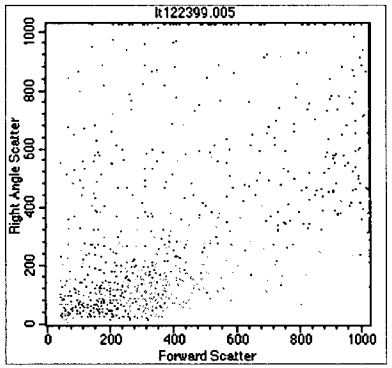


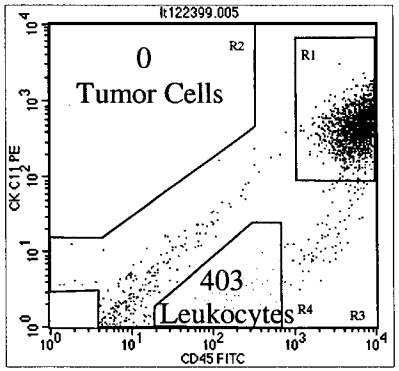
Figure 4

Flow Cytometric Analysis of EPCAM FF Selected Cells from Blood
Threshold on forward light scatter & gate excluding fluorescent negative events.

Unspiked 2 ml Blood Sample

a.





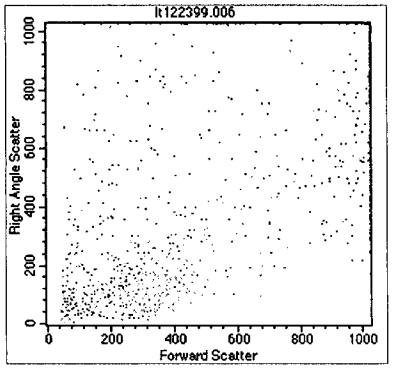
b.

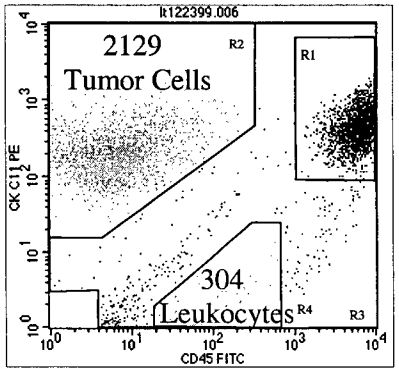
2647 Control Cells

(2647/5000) x 2ml = 1.1 ml
Blood Analyzed
0 Tumor Cells Detected

Spiked 2 ml Blood Sample

c.





d.

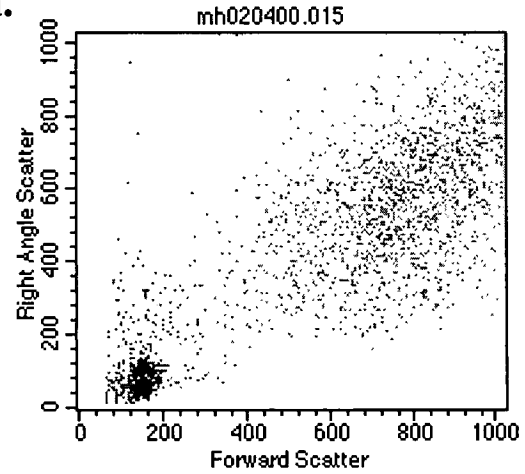
2667 Control Cells

(2667/5000) x 2ml = 1.1 ml
Blood Analyzed
2129 Tumor Cells Detected

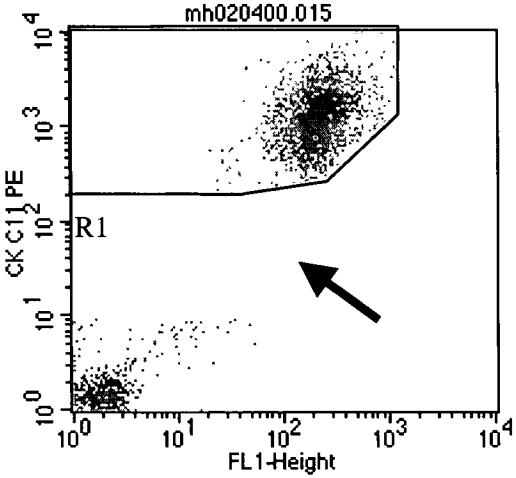
2129 = 1996 cells
(2667/5000) x 2 /ml blood

Figure 5

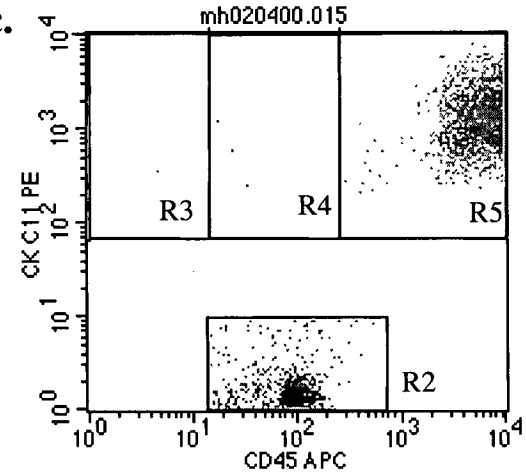
a.



b.



c.



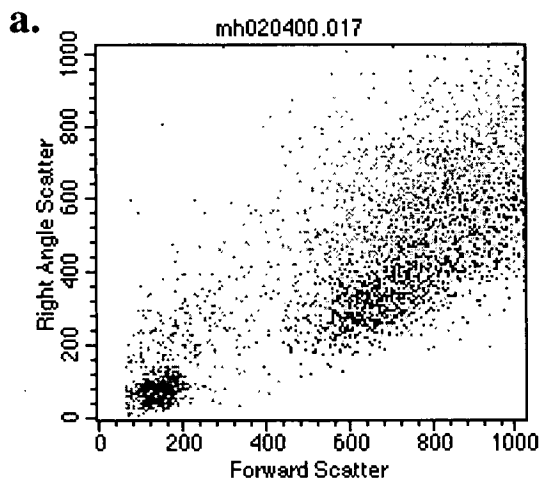
d.

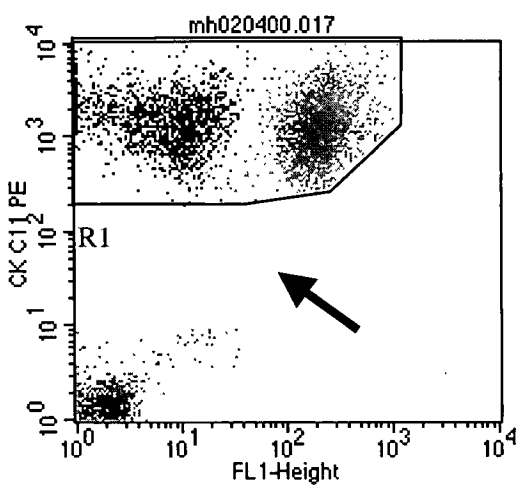
File: mh020400.015 Acquisition Date: 9-Feb-0
Gated Events: 3170 Total Events: 4560

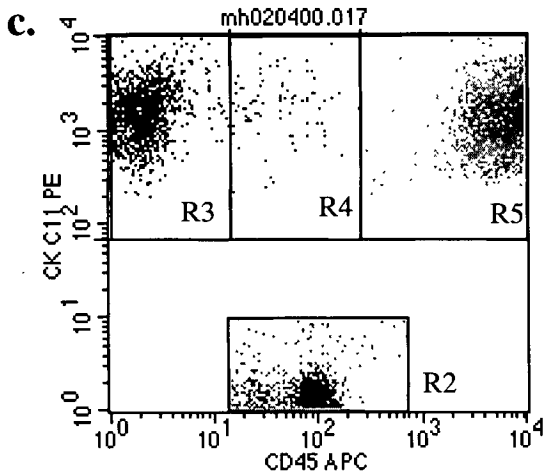
Gate	Events	% Gated	% Total
tumor	0	0.00	0.00
possible tumor	3	0.09	0.07
control cells	2023	63.82	44.36
leukocytes	1144	36.09	25.09
cells	3170	100.00	69.52

Figure 6

7/7







d.

File: mh020400.017 Acquisition Date: 9-Feb-0
Gated Events: 5400 Total Events: 6945

Gate	Events	% Gated	% Total
tumor	1644	30.44	23.67
possible tumor	82	1.52	1.18
control cells	2042	37.81	29.40
leukocytes	1632	30.22	23.50
cells	5400	100.00	77.75

Figure 7